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Factors produced by activated leukocytes alter renal epithelial cell differentiation

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Factors produced by activated leukocytes alter renal epithelial cell differentiation. The development of tubulointerstitial fibrosis in inflammatory renal diseases has been linked to disease progression to end-stage renal failure. Understanding the interactions of the factors influencing inflammation and activating the fibrotic process, that is, the inflammatory infiltrate and the resident proximal tubular cells, may lead to a determination of the mechanisms that regulate tubulointerstitial fibrosis. We used an *in vitro* model of human proximal tubule cells that were stimulated with supernatant from activated peripheral blood mononuclear cells (leukocytes) to study the alterations in cellular phenotype, and examined the signaling pathways mediating epithelial-fibroblast like transdifferentiation. Our hypothesis of the proposed sequence of events leading to tubulointerstitial fibrosis is explained.

Tubulointerstitial fibrosis is the final common pathway for most inflammatory renal diseases that can progress to end-stage renal failure, and the level of fibrosis correlates closely with the loss of renal function observed in patients with chronic glomerular disease [1, 2]. The tubulointerstitial inflammatory infiltrate may play a major role in the pathogenesis of the tubular and interstitial lesions observed in chronic glomerulonephritis. Many studies have shown a strict correlation between tubular atrophy, interstitial fibrosis, and the extent of the interstitial infiltrate [3, 4]. This infiltrate has been shown to consist of an inflammatory population of cells that is predominantly monocytes and T lymphocytes. It has been suggested that these inflammatory cells are recruited into the inflamed interstitium usually as a consequence of glomerular-derived inflammatory mediators that diffuse to the tubule stimulating its immune activation [5]. Following these glomerular-derived stimuli, tubular cells have been shown to express chemokines such as monocyte chemoattractant protein-1 (MCP-1), RANTES, and interleukin-8 (IL-8), adhesion molecules such as vascular cell adhesion molecule-1 (VCAM-1), and intercellular adhesion molecule-1 (ICAM-1), all of which serve to attract additional inflammatory cells to the interstitium [6]. The recruited mononuclear cells can then release an array of inflammatory mediators that can injure tubular cells and activate interstitial fibroblasts. The production of fibrotic cytokines and the excessive synthesis of extracellular matrix components by cells within the tubulointerstitium ensue, culminating in the development of tubulointerstitial injury with subsequent fibrosis and disease progression [7]. In this study, human proximal tubular epithelial cells (HPTs) were stimulated with supernatant from activated peripheral mononuclear cells, and alterations in cellular phenotype were assessed in an attempt to examine the possible consequences of interactions between these major players in tubulointerstitial disease, namely, the inflammatory infiltrate and the resident proximal tubular cells.

Primary HPTs and the HK-2 proximal tubular cell line [8] were exposed to a supernatant from activated peripheral blood mononuclear cells (aPBMCs), and alterations in the phenotype were assessed in terms of morphology and marker protein expression. Alterations in transepithelial resistance were used as a measure of the epithelial barrier function. Both HPT and HK-2 cells treated with the supernatant from activated mononuclear cells showed alterations in morphology to a more elongated fibroblast-like cell type, as evidenced by phase contrast microscopy (Fig. 1) and supported by transmission electron microscopy. These alterations in cellular morphology were reversible upon removal of the immune stimulus present in the supernatant from activated mononuclear cells. Treatment of HPT cells with aPBMCs for 48 hours caused a significant decrease in transepithelial resistance to 36 $\pm$ 4% ($P < 0.001$) of control value (abstract; Healy et al, *Am Soc Nephrol* 8:474A, 1997). In addition, the expression of the epithelial junctional proteins e-cadherin and occludin was down-regulated,

Key words: tubulointerstitial disease, fibrosis, inflammation, proximal tubule cells, end-stage renal failure.

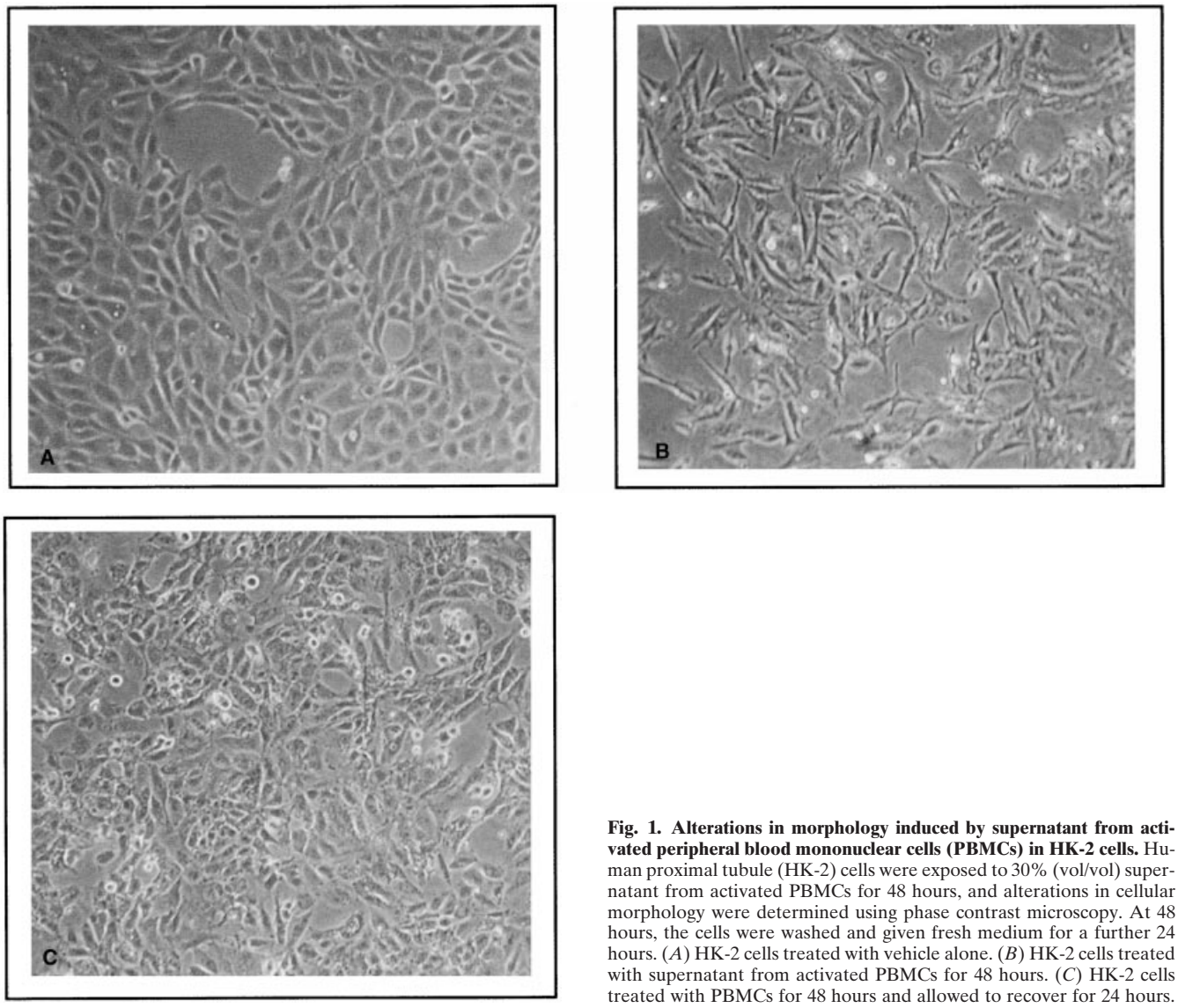

Fig. 1. Alterations in morphology induced by supernatant from activated peripheral blood mononuclear cells (PBMCs) in HK-2 cells. Human proximal tubule (HK-2) cells were exposed to 30% (vol/vol) supernatant from activated PBMCs for 48 hours, and alterations in cellular morphology were determined using phase contrast microscopy. At 48 hours, the cells were washed and given fresh medium for a further 24 hours. (*A*) HK-2 cells treated with vehicle alone. (*B*) HK-2 cells treated with supernatant from activated PBMCs for 48 hours. (*C*) HK-2 cells treated with PBMCs for 48 hours and allowed to recover for 24 hours.

and the mesenchymal marker vimentin was up-regulated in a time-dependent manner in treated cells. Preliminary findings have also shown that parallel to these changes in epithelial phenotype sustained activation of both the p38 and p42/44 mitogen-activated protein (MAP) kinases occurs. Therefore, some currently unknown factors produced by the activated mononuclear cells are capable of inducing this alteration in epithelial phenotype, which exhibits characteristics of a phenomenon known as "transdifferentiation."

Transdifferentiation is a process whereby one cellular phenotype is lost, whereas another is gained by manipulation of their environment [9]. It was widely assumed that interstitial fibroblasts were the main culprits for extracellular matrix synthesis in tubulointerstitial fibrosis. In recent studies performed in cultured cells and experimental nephropathies, it has been hypothesized that renal fibroblasts can be produced at sites of injury by conversion from tubular epithelial cells by this process of transdifferentiation [10]. This hypothesis was verified in clinical studies whereby immunohistochemical analysis of biopsy sections from patients with glomerulonephritis demonstrated that some tubular epithelial cells were being altered to collagen-producing fibroblast cells in the diseased kidney (abstract; Rastaldi et al, *J Am Soc Nephrol* 8:525A, 1997) [11]. The degree of transdifferentiation correlated with the severity of tubular damage and subsequent disease progression. Strutz et al identified a specific fibroblast marker protein that they termed FSP1 [12]. Mouse tubular epithelium transfected with FSP1 began to show signs of transdifferentiation to a more fibroblast-like phenotype associated with a loss of

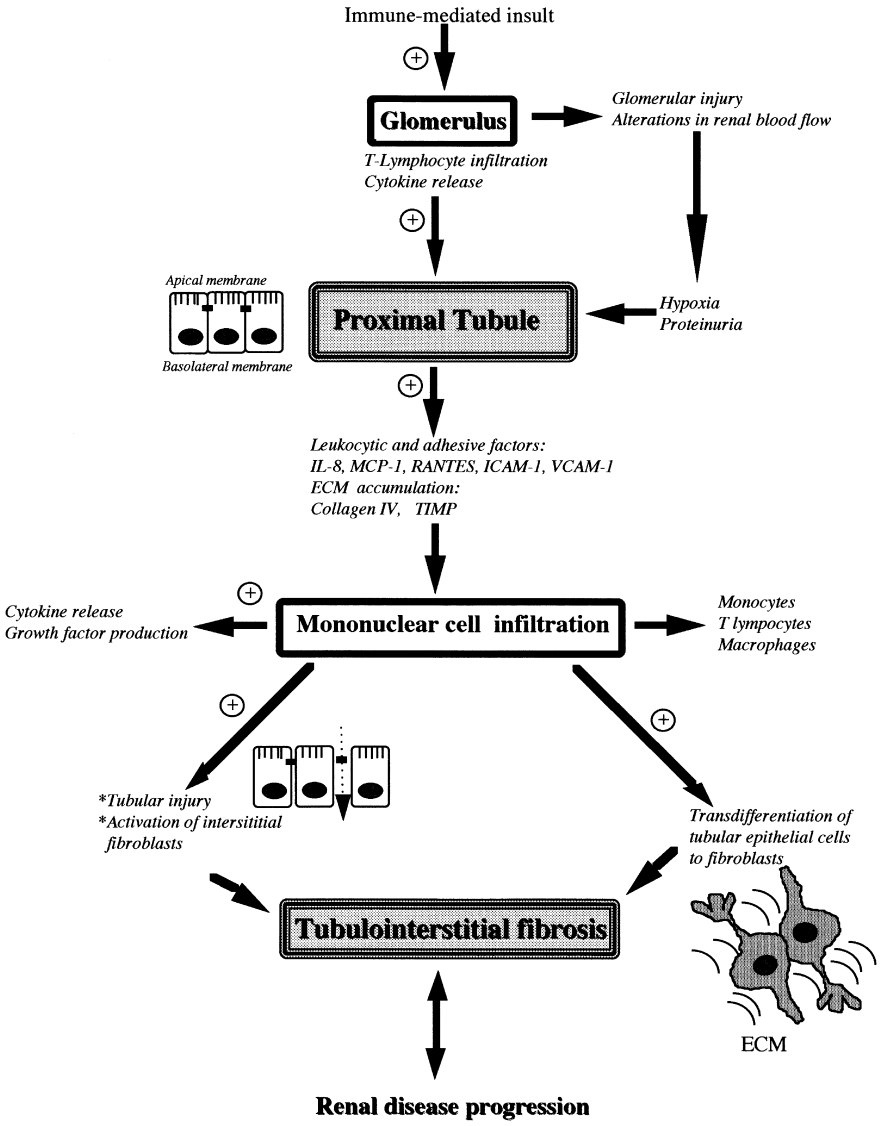

Fig. 2. Proposed sequence of events that lead to tubulointerstitial fibrosis and eventually to progressive renal disease.

cytokeratin expression and a gain of vimentin expression. Okada et al demonstrated that mouse renal proximal tubule epithelial cells exposed to the cytokines epidermal growth factor (EGF) and transforming growth factor-β (TGF-β) transdifferentiate to fibroblast-like cells with increased expression of collagen, vimentin, FSP-1 and α-smooth muscle actin [13]. FSP-1 antisense oligomers blocked this cytokine-induced epithelial transformation, indicating that FSP1 expression is an important early event in the pathway toward transdifferentiation. MAP kinase (MAPK) activation has been shown to mediate cellular differentiation in a number of cell systems. Extracellular signal-related kinase-1 and -2 (ERK1 and ERK2) represent a subfamily of MAP kinases. Stable expression of the upstream MAPK/ERK in renal epithelial cells also induces epithelial transdifferentiation to fibroblast-like cells [14]. This is interesting in view of the fact that we have observed sustained MAP kinase activation parallel to the alterations in epithelial phenotype induced by aPBMC supernatant in HPTs (unpublished observations).

Studies by other laboratories have also examined the consequences of cell–cell interactions in renal disease. Dialyzed supernatants from lymphocytes from nephritic animals were shown to stimulate both an increase in fibroblast proliferation and collagen production. Supernatants from control animal lymphocyte cultures conversely contained inhibitors of these two processes [15]. PBMC culture supernatants from patients with IgA nephropathy caused an increase in intercellular adhesion molecule-1 expression on human mesangial cells in culture [16]. An epithelial–lymphocyte interaction is not unique to the kidney. Taylor et al have shown that coculture of T84 colonic epithelial cells with intraepithelial

lymphocytes induced decreases in the epithelial barrier function, as indicated by decreases in the transepithelial resistance [17].

These results indicate that cross-talk between resident renal cells and the infiltrating inflammatory cells may be crucial in the development of tubulointerstitial fibrosis (a proposed hypothesis is shown in Fig. 2). Further delineation of these signaling pathways that mediate this epithelial-fibroblast-like transdifferentiation could aid in the understanding of the molecular mechanisms that regulate tubulointerstitial fibrosis.

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